Three New Dimeric Benzofuran Derivatives from the Roots of *Ligularia stenocephala* MATSUM. et KOIDZ.

Kazuki TOYODA, Yasunori YAOITA, and Masao KIKUCHI*

Tohoku Pharmaceutical University; 4–4–1 Komatsushima, Aoba-ku, Sendai, Miyagi 981–8558, Japan.

Received July 6, 2005; accepted August 24, 2005

Three new dimeric benzofuran derivatives, ligulacephalins A (1), B (2) and C (3), were isolated from the roots of *Ligularia stenocephala* MATSUM. ET KOIDZ. (Compositae) together with three known compounds, 5,6-dimethoxy-2-isopropenylbenzofuran (4), euparin (5) and (R)-(−)-hydroxytremetone (6). The structures of the new compounds were determined by spectroscopic evidence. The chiral HPLC analysis demonstrated that 1—3 occurred as a racemate. The absolute configurations of each enantiomer from 1—3 were elucidated on the basis of circular dichroism (CD) data.

Key words  *Ligularia stenocephala*; Compositae; dimeric benzofuran derivative

*Ligularia stenocephala* MATSUM. et KOIDZ. (Compositae) has long been used as a Chinese folk medicine in the treatment of edema and scrofula.1) The constituents of *L. stenocephala* have been previously investigated and shown to contain benzofuran derivatives.1—5) Here, we report the isolation and structural elucidation of three new dimeric benzofuran derivatives, ligulacephalins A (1), B (2) and C (3), together with three known compounds from the roots of *L. stenocephala*. The known compounds were identified as 5,6-dimethoxy-2-isopropenylbenzofuran (4),2) euparin (5)6) and (R)-(−)-hydroxytremetone (6),7) respectively, by comparison of their spectroscopic data with those previously described in the literature.

Ligulacephalin A (1) was obtained as an amorphous powder. The molecular formula was determined to be C_{26}H_{28}O_{6} by high-resolution (HR)-electron ionization (EI)-MS, indicating thirteen degrees of unsaturation. The UV absorption maxima at 206, 249sh, 255 and 295 nm indicated the presence of a benzofuran ring.8) The presence of 13 carbons and 14 hydrogens shown in the NMR spectra, and an intense ion peak at m/z 218 (M^{+}/2, 100%) in the EI-MS, suggested the symmetrical nature of 1. The $^1$H- and $^{13}$C-NMR data (vide Experimental) indicated that 1 possessed one methyl group, one methylene group, two methoxyl groups, one quaternary carbon and a benzofuran skeleton. Interpretation of the $^1$H-detected heteronuclear multiple-bond coherence (HMBC) and nuclear Overhauser effect correlation spectroscopy (NOESY) data led to the half-unit of the molecule (Fig. 1). Based on the molecular formula, C-10 must form a cyclobutane ring with C-10′ or C-12′. The coupling pattern and the constants for the methylene protons [J_{AX} = 11.3 Hz, J_{AX}/H_{11032}/H_{11005} = 9.5 Hz, J_{XX}/H_{11032}/H_{11005} = 3.8 Hz] suggested a head-to-head dimeric structure.9,10) Thus the gross structure of ligulacephalin A was determined to be as shown in 1. The relative stereochemistry was determined as follows. In the long-range $^1$H–$^1$H shift correlation spectroscopy (H–H COSY) spectrum, a W-type coupling was observed between H-11′ and H-12′α, indicating their anticoplanar orientation (Fig. 2). The NOESY
cross peaks observed between H₃-11′ and H-3, H₃-11 and H-12′β and H₃-11′ and H-12β implied a trans relationship of two 5,6-dimethoxybenzofuran rings at C-10 and C-10′. Therefore, the structure of 1 was concluded to be as shown in Fig. 2. Despite the presence of two asymmetric carbons at C-10 and C-10′, the specific rotation of 1 was almost zero, suggesting its racemic nature. This was proved by chiral HPLC, in which 1 was separated into two peaks in a ratio of 1:1 [peak A, (+)-1: [α]₀°⁹ +146.1° (MeOH); peak B, (−)-1: [α]₀°⁹ -151.2° (MeOH)], both of which gave ¹H-NMR spectra identical to those of the starting material. The circular dichroism (CD) spectra of (+)-1 and (−)-1 exhibited mirror images (Fig. 3), confirming their enantiomeric relationship. The absolute configuration of (+)-1 was elucidated by the CD exciton chirality method. ¹¹) The sign of the first Cotton effect was positive ([λ]max 263.2 nm (Δε +32.9°)), while that of the second one ([λ]max 246.5 nm (Δε -29.8°)) was negative (Fig. 3), indicating that the chirality between two 5,6-dimethoxybenzofuran rings at C-10 and C-10′ should be clockwise. Thus the absolute configurations at C-10 and C-10′ of (+)-1 were assigned as S and S, respectively (Fig. 3). Therefore, the absolute configurations at C-10 and C-10′ of (−)-1 were assigned as R and R, respectively. Accordingly, compounds (+)-1 and (−)-1 were concluded to be (S,S)-(−)- and (R,R)-(−)-ligulacephalin A, respectively.

Ligulacephalin B (2) had the molecular formula C₂₅H₂₆O₇ on the basis of HR-ESI-MS, with 13 carbon and 14 hydrogens were observed and remaining 13 carbons and 14 hydrogens were not detected, it was suggested that 2 possessed a symmetrical structure. Compound 2 showed a very similar signal pattern to that of 1 in the ¹³C-NMR spectrum. However, the quaternary carbon signal (δ 46.8) assignable to C-10 (C-10′) of 1 was shifted down to δ 83.6 in 2, suggesting that the cyclobutane ring of 1 was replaced by a tetrahydrofuran ring. This was also supported by the molecular formula and the unsaturation degree. The relative stereochemistry of 2 was determined as follows. In the long-range ¹H–¹H COSY spectrum, a W-type coupling was observed between H₃-11 and H-12′α (Fig. 2). The NOESY cross peaks observed between H₃-11′ and H-3, H₃-11′ and H-12β, and H₃-11′ and H-12β implied a trans relationship of the two 5,6-dimethoxybenzofuran rings at C-10 and C-10′. Thus the structure of ligulacephalin B was concluded to be as shown in Fig. 2. As the specific rotation of 2 showed almost zero, 2 is presumably a racemate at the chiral centers C-10 and C-10′. This was proved by chiral HPLC, in which 2 was separated into two peaks in a ratio of 1:1 [peak A, (+)-2: [α]₀°⁹ +65.5° (MeOH); peak B, (−)-2: [α]₀°⁹ -67.6° (MeOH)]. The CD spectra of (+)-2 and (−)-2 exhibited mirror images (Fig. 4). In the CD spectrum of (+)-2, the sign of the first Cotton effect was positive ([λ]max 258.1 nm (Δε +10.6°)), while that of the second one ([λ]max 242.9 nm (Δε -1.4°)) was negative (Fig. 4), indicating that the absolute configurations at C-10 and C-10′ of (+)-2 were assigned as R and R, respectively (Fig. 4). Therefore, the absolute configurations at C-10 and C-10′ of (−)-2 were assigned as S and S, respectively. Accordingly, compounds (+)-2 and (−)-2 were concluded to be (R,R)-(−)- and (S,S)-(−)-ligulacephalin B, respectively.

Ligulacephalin C (3) was assigned the molecular formula C₂₅H₂₅O₆ using HR-ESI-MS. The UV spectrum showed absorptions characteristic of a benzofuran ring. ²⁸) The ¹H- and ¹³C-NMR data indicated that 3 possessed one methyl group,
two methylenes, four methoxyl groups, one exomethylene, one quaternary carbon and two benzofuran rings. By $^1$H-$^1$H COSY, HMBC and NOESY spectra, the structure of ligulacephalin C was deduced to be as shown in Fig. 5. Despite the presence of an asymmetric carbon at C-10, the specific rotation of 3 was almost zero, suggesting its racemic nature. This was proved by chiral HPLC, in which 3 was separated into two peaks in a ratio of 1:1 [peak A, (+)-3: $[\alpha]_{D}^{24} + 89.3^\circ$ (MeOH); peak B, (−)-3: $[\alpha]_{D}^{24} - 80.0^\circ$ (MeOH)]. The CD spectra of (+)-3 and (−)-3 exhibited mirror images (Fig. 6), confirming their enantiomeric relationship. The absolute configuration at C-10 of (+)-3 was established on the basis of NOESY and CD spectroscopic evidence (Fig. 6). The NOESY cross peak observed between H$_{11}$-11 methyl group and H-4 implied a pseudo-equatorial orientation of the methyl group at C-10 and a pseudo-axial orientation of the 5,6-dimethoxybenzofuran ring at C-10. Further, (+)-3 gave a positive Cotton effect at 320.5 nm ($\Delta \epsilon + 2$) and a negative Cotton effect at 294.8 nm ($\Delta \epsilon - 5.3$), indicating that the absolute configuration of C-10 should be S. Thus the absolute configuration at C-10 of (−)-3 was assigned as R. Accordingly, compounds (+)-3 and (−)-3 were concluded to be (S)-(−) and (R)-(−)-ligulacephalin C, respectively.

A possible formation of 1–3 is proposed in Fig. 7. Compounds 1–3 are presumably formed from 5,6-dimethoxy-2-isopropenylbenzofuran (4), the major benzofuran derivative of the roots of *L. stenocephala*. Compounds 1–3 occurred as a racemate, suggesting that they are artifacts or that they are formed in the plant cells without the participation of enzymes.

### Experimental

#### General Procedures

Optical rotations were determined using a JASCO DIP-360 digital polarimeter. CD spectra were measured on a JASCO J-720 spectropolarimeter. UV spectra were recorded with a Beckman DU-64 spectrophotometer. $^1$H- and $^13$C-NMR spectra were recorded on JEOL JNM-LA 600 (600 and 150 MHz, respectively) and JEOL JNM-LA 400 (400 and 100 MHz, respectively) spectrometers. Chemical shifts are given on a $\delta$ (ppm) scale, with tetramethylsilane as internal standard. El-MS and HR-ESI-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on a Kieselgel 60 (230–400 mesh, Merck). HPLC was carried out on a Tosoh HPLC system (pump, CCPD; detector, RI-8010).

#### Plant Material

The roots of *Ligularia stenocephala* (0.2 kg) were extracted three times with Et$_2$O at room temperature over a 2 week period. The Et$_2$O extract (6.4 g) was chromatographed on a silica gel column using hexane–iso-PrOH (90 : 1); flow rate, 1.0 ml/min. The separation of 1 into its enantiomers [(+)–1 (0.5 mg, $t_{R} 18.6$ min), (−)–1 (0.4 mg, $t_{R} 20.2$ min)] was achieved by preparative HPLC using a chiral column chromatograph (column, Chiralpak AD-H (4.6 mm i.d. ×25 cm, Daicel Chemical Industries, Ltd.); mobile phase, n-hexane–iso-ProOH (90 : 5); flow rate, 1.0 ml/min). The separation of 2 into its enantiomers [(+)–2 (0.6 mg, $t_{R} 14.7$ min), (−)–2 (0.5 mg, $t_{R} 16.6$ min)] was achieved by preparative HPLC using a chiral column chromatograph (column, Chiralcel OD (4.6 mm i.d. ×25 cm, Daicel Chemical Industries, Ltd.); mobile phase, n-hexane–iso-ProOH (9 : 1); flow rate, 1.0 ml/min). The separation of 3 into its enantiomers [(+)–3 (1.0 mg, $t_{R} 20.6$ min), (−)–3 (0.6 mg, $t_{R} 21.7$ min)] was achieved by preparative HPLC using a chiral column chromatograph (column, Chiralpak AD-RH (4.6 mm i.d. ×15 cm, Daicel Chemical Industries, Ltd.); column temperature, 40 °C; mobile phase, CH$_3$CN–H$_2$O (1 : 1); flow rate, 1.0 ml/min).

Ligulacephalin A (1): Amorphous powder. $[\alpha]_{D}^{24}$ $\pm 0^\circ$ ($c$=0.10, MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\epsilon$): 206 (4.6), 249 (4.3), 255 (4.3), 295 (4.3). EI-MS $m/z$ (rel. int. %): 436 (M$^+$, 11), 421 (10), 210 (100), 203 (24). HR-ESI-MS $m/z$: 436.1915 (M$^+$, Caled for C$_{26}$H$_{28}$O$_6$: 436.1886). $^1$H-NMR (600 MHz, CD$_3$OD) $\delta$: 1.28 (6H, s, CH$_3$-11, CH$_3$-11’), 1.94 (2H, AA’XX’ type, H-12r, H-12’b), 2.78 (2H, AA’XX’ type, H-12b, H-12’a) [Coupling constants, $J_{AX}=7.3$ Hz, $J_{XX}=9.5$ Hz, $J_{AB}=9.0$ Hz, $J_{AB}=-3.8$ Hz], 3.87 (6H, s, CH$_3$O-6, CH$_3$O-6’), 6.52 (2H, d, $J=0.7$ Hz, H-3, H-3’), 7.10 (2H, s, H-4, H-4’), 7.18 (2H, s, H-7, H-7’), 1.36 (1H, m, H-11, H-11’), 2.48 (1H, m, H-11, H-11’). $^1$C-NMR (150 MHz, CD$_3$OD) $\delta$: 23.5 (C-1, C-1’), 25.7 (C-2, C-2’), 26.8 (C-3, C-3’), 70.2 (C-4, C-4’), 72.2 (C-5, C-5’), 103.7 (C-6, C-6’), 112.0 (C-9, C-9’), 122.3 (C-9, C-9’), 147.8 (C-5, C-5’), 148.9 (C-6, C-6’), 151.1 (C-8, C-8’), 163.4 (C-2, C-2’).
Fig. 6. CD Spectra of (S)(+)-3 (——) and (R)(−)-3 (----) in MeOH and Selected NOESY (Dotted-Line Arrows) Correlation of (S)(+)-3

Fig. 7. Possible Pathway for 1—3 from the Benzofuran 4

(S,S)-(+)—Ligulacephalin A [(+)-1]: Amorphous powder. \([\alpha]_D^{25} +146.1^\circ\) (c=0.05, MeOH). CD \(\lambda_{em} (c=1.36\times 10^{-3} M, MeOH) (\Delta\lambda): 309.0 (23.0), 263.2 (+32.9), 266.5 (−29.8), 217.3 (+4.5), 202.1 (−30.4).

(R,R)-(−)—Ligulacephalin A [(−)-1]: Amorphous powder. \([\alpha]_D^{29} −151.2^\circ\) (c=0.05, MeOH). CD \(\lambda_{em} (c=2.43\times 10^{-3} M, MeOH) (\Delta\lambda): 309.5 (−25.4), 263.4 (−28.4), 246.8 (+20.4), 217.2 (−6.1), 203.2 (+22.3).

Ligulacephalin B (2): Amorphous powder. \([\alpha]_D^{25} ±0^\circ\) (c=0.22, MeOH). UV \(\lambda_{max} (MeOH) nm (log e): 207 (4.7), 247 (4.4), 253 (4.4), 294 (4.2). ELMs \(m/z\) (rel. int. %): 452 (M+1, 88), 437 (100), 232 (58), 218 (42). HR-El-MS \(m/z\): 452.1834 (M+, Caled for C23H22O7: 452.1835). 1H-NMR (400 MHz, CD3OD) \(\delta\): 1.72 (6H, s, CH3-11, CH3-11'), 2.27 (2H, m, H-12α, H-12β), 2.59 (2H, m, H-12α, H-12β), 3.36 (6H, s, CH3O-5, CH3O-5'), 3.88 (6H, s, CH3O-6, CH3O-6'), 6.64 (2H, d, J=1.0 Hz, H-3, H-3'), 7.10 (2H, s, H-4, H-4'), 7.14 (2H, s, H-7, H-7'). 13C-NMR (100 MHz, CD3OD) \(\delta\): 27.3 (C-11, C-11'), 38.6 (C-12, C-12'), 56.9, 57.2 (CH3O-5, CH3O-5', CH3O-6, CH3O-6'), 83.6 (C-10, C-10'), 96.9 (C-7, C-7'), 103.0 (C-3, C-3'), 104.6 (C-4, C-4'), 121.8 (C-9, C-9'), 148.0 (C-5, C-5'), 149.4 (C-6, C-5'), 151.2 (C-8, C-8'), 162.3 (C-2, C-2').

(R,R)-(−)−Ligulacephalin B [(+)-2]: Amorphous powder. \([\alpha]_D^{25} +65.5^\circ\) (c=0.06, MeOH). CD \(\lambda_{em} (c=2.41\times 10^{-3} M, MeOH) (\Delta\lambda): 305.4 (−5.8), 258.1 (+10.6), 242.9 (−1.4), 214.3 (+6.1).

(S,S)-(−)−Ligulacephalin B [(−)-2]: Amorphous powder. \([\alpha]_D^{29} −67.6^\circ\) (c=0.05, MeOH). CD \(\lambda_{em} (c=1.85\times 10^{-3} M, MeOH) (\Delta\lambda): 302.4 (−5.9), 258.2 (−13.0), 243.9 (+2.0), 216.4 (−4.4).

Ligulacephalin C (3): Amorphous powder. \([\alpha]_D^{29} ±0^\circ\) (c=0.40, MeOH). UV \(\lambda_{max} (MeOH) nm (log e): 207 (4.6), 247 (4.2), 253 (4.2), 299 (4.3), 303 (4.3), 320 (4.2). ELMs \(m/z\) (rel. int. %): 434 (M+, 100), 419 (67), 219 (30), 217 (18). HR-El-MS \(m/z\): 434.1747 (M+, Caled for C23H22O7: 434.1729). 1H-NMR (500 MHz, CD3OD) \(\delta\): 1.82 (3H, s, CH3-11), 1.92 (1H, m, H-12α), 2.42 (1H, m, H-12β), 2.54 (1H, m, H-12β), 2.63 (1H, m, H-12β), 3.61 (3H, s, CH3O-5, CH3O-5'), 3.81 (6H, s, CH3O-5, CH3O-6), 3.86 (3H, s, CH3O-6'), 6.35 (1H, s, H-3), 6.72 (1H, s, H-4), 7.02 (1H, s, H-4), 7.04 (1H, s, H-7), 7.13 (1H, s, H-7'). 13C-NMR (150 MHz, CD3OD) \(\delta\): 25.4 (C-11), 29.0 (C-12), 38.6 (C-12), 39.2 (C-10), 56.79, 56.82, 57.0, 57.1 (CH3O-5, CH3O-5', CH3O-6, CH3O-6'), 96.7, 96.9 (C-7, C-7'), 104.0, 104.1 (C-3, C-4'), 104.3 (C-4), 106.5 (C-11), 120.5, 121.8 (C-9, C-9', C-9'), 135.4 (C-10'), 147.6, 147.8 (C-5, C-5'), 149.1, 150.0 (C-6, C-6'), 151.1, 151.2 (C-8, C-8'), 151.9 (C-2', C-2'), 162.9 (C-2).

(S)(+)-Ligulacephalin C [(+)-3]: Amorphous powder. \([\alpha]_D^{25} +89.3^\circ\) (c=0.06, MeOH). CD \(\lambda_{em} (c=3.22\times 10^{-3} M, MeOH) (\Delta\lambda): 320.5 (−2.4), 294.8 (−5.3), 255.6 (−5.5), 248.6 (+4.3), 206.8 (+30.3).

(R)(−)-Ligulacephalin C [(−)-3]: Amorphous powder. \([\alpha]_D^{25} −80.0^\circ\) (c=0.05, MeOH). CD \(\lambda_{em} (c=2.88\times 10^{-3} M, MeOH) (\Delta\lambda): 319.8 (−2.1), 294.5 (+5.2), 255.2 (−5.5), 246.9 (−4.3), 207.1 (−30.2).

Acknowledgments We are grateful to Mr. S. Sato and Mr. T. Matsuki of this university for measurement of the mass and NMR spectra.

References